Claims

1. A highly sensitive real-time RT-PCR capable of specifically detecting the expression of more than one MAGE gene, wherein reverse transcription of the corresponding MAGE transcripts is carried out simultaneously in a single cDNA-synthesis reaction.

2. The method of claim 1, wherein the MAGE genes are selected from the functional genes of MAGE subfamilies A, B and/or C.

3. The method of claim 1 or 2, wherein the selected MAGE genes comprise MAGE-A 1, 2, 3, 4, 6, 10 and/or 12.

4. The method of any one of the preceding claims, wherein at least one primer for reverse transcription of MAGE mRNA is selected from the following groups of oligonucleotides:

(A)

primer	sequence (5' - 3')
MgRT1a	CCA GCA TTT CTG CCT TTG TGA
MgRT1b	CCA GCA TTT CTG CCT GTT TG
MgRT2	CAG CTC CTC CCA GAT TT
MgRT3a	ACC TGC CGG TAC TCC AGG
MgRT3b	ACC TGC CGG TAC TCC AGG TA
MgRT4	GCC CTT GGA CCC CAC AGG AA
MgRT5a	AGG ACT TTC ACA TAG CTG GTT TCA
MgRT5b	GGA CTT TCA CAT AGC TGG TTT C
MgRT6	TTT ATT CAG ATT TAA TTT C

(B)

primer	sequence (5' - 3')
Mg1_RT1	CAA GAG ACA TGA TGA CTC TC
Mg1_RT2	TTC CTC AGG CTT GCA GTG CA
Mg1_RT3	GAG AGG AGG AGG AGG TGG C
Mg1_RT4	GAT CTG TTG ACC CAG CAG TG
Mg1_RT5a	CAC TGG GTT GCC TCT GTC
Mg1_RT5c	CTG GGT TGC CTC TGT CGA G
Mg1_RT5d	GGG TTG CCT CTG TCG AGT G
Mg1_RT5e	GGC TGC TGG AAC CCT CAC
Mg1_RT6	GCT TGG CCC CTC CTC TTC AC
Mg1_RT7	GAA CAA GGA CTC CAG GAT AC

5. The method of any one of the preceding claims, wherein in addition to the reverse transcription of MAGE transcripts reverse transcription of a calibrator mRNA is simultaneously carried out in the same single cDNA-synthesis reaction followed by PCR-amplification of MAGE- and calibrator cDNAs.

6. The method of claim 5, wherein the calibrator mRNA is porphobilinogen desaminase (PBGD), glyceraldehyd-3-phospat dehydrogenase (GAPDH), beta-2-microglobin or beta-actin.

7. The method of claim 6, wherein the primer for reverse transcription of PBGD mRNA is selected from the following group of oligonucleotides:

primer	sequence (5' - 3')
PBGD_RT2	CAT ACA TGC ATT CCT CAG GGT
PBGD_RT3	GAA CTT TCT CTG CAG CTG GGC
PBGD_RT4	TGG CAG GGT TTC TAG GGT CT
PBGD_RT10a	GGT TTC CCC GAA TAC TCC TG
PBGD_RT10d	TTG CTA GGA TGA TGG CAC TG
PBGD_RT12b	CCA AGA TGT CCT GGT CCT TG
PBGD_RT12c	CAG CAC ACC CAC CAG ATC
PBGD_RT12d	AGA GTC TCG GGA TCG TGC
PBGD_RT12e	AGT CTC GGG ATC GTG CAG
PBGD_RT12f	TCT CGG GAT CGT GCA GCA
PBGD_RT12g	ATG CAG CGA AGC AGA GTC T
PBGD_RT12h	CCT TTC AGC GAT GCA GCG
PBGD_RT13a	GTA TGC ACG GCT ACT GGC
PBGD_RT14a	GCT ATC TGA GCC GTC TAG AC
PBGD_RT15a	AAT GTT ACG AGC AGT GAT GC
PBGD_RT15b	TGG GGC CCT CGT GGA ATG
PBGD_RT15e	CAG TTA ATG GGC ATC GTT AAG
PBGD_RT15f	ATC TGT GCC CCA CAA ACC AG
PBGD_RT15g	GGC CCG GGA TGT AGG CAC
PBGD_RT15h	GGT AAT CAC TCC CCA GAT AG
PBGD_RT15i	CTC CCG GGG TAA TCA CTC
PBGD_RT15j	CAG TCT CCC GGG GTA ATC
PBGD_RT15k	TGA GGA GGC AAG GCA GTC
PBGD_RT15l	GGA TTG GTT ACA TTC AAA GGC

8. The method of claims 5 to 7, wherein the PCR-primers for amplification of PBGD-cDNA comprise oligonucleotides selected from the following groups:

PBGD sense primer	sequence (5' - 3')
hu_PBGD_se	AGA GTG ATT CGC GTG GGT ACC
PBGD_8	GGC TGC AAC GGC GGA AGA AAA C
PBGD_ 8_F	TGC AAC GGC GGA AGA AAA C
PBGD_ATG-Eco	ATG TCT GGT AAC GGC AAT GC

PBGD antisense primer	sequence (5' - 3')
PBGD_3	TTG CAG ATG GCT CCG ATG GTG AA
PBGD_3.1_R	GGC TCC GAT GGT GAA GCC
PBGD_R	TTG GGT GAA AGA CAA CAG CAT C

9. The method of claim 8, wherein oligonucleotides hu PBGD se and PBGD 3.1 R or hu PBGD se and PBGD R are used as primer pairs for PCR-amplification of PBGD-cDNA.

10. The method of any one of the preceding claims, wherein in total not more than two different oligonucleotides are used as primers for reverse transcription in the cDNA-synthesis reaction.

11. The method of claim 10, wherein oligonucleotides MgRT3a and/or Mg1 RT5a are used as primers for reverse transcription in the cDNA-synthesis reaction.

12. The method of claim 10, wherein oligonucleotides MgRT3a and PBGD RT15b are used as primers for reverse transcription in the cDNA-synthesis reaction.

13. The method of any one of the preceding claims, wherein the MAGE- and/or the calibrator-PCR are nested or semi-nested PCRs.

14. The method of any one of the preceding claims, wherein PCR-primers are used comprising pairs of oligonucleotides specifically amplifying only a single member of the selected group of MAGE genes, respectively.

15. The method of any one of the preceding claims, wherein PCR-primers are used comprising pairs of oligonucleotides comprising pairs of PCR-primers amplifying more than one member of the selected group of MAGE genes, respectively.

16. The method of any one of the preceding claims, wherein the PCR-primers for amplification of MAGE-cDNA comprise oligonucleotides selected from one of the following groups:

(C)

PCR-primer	sequence (5' - 3')
MAGE-A1	GTA GAG TTC GGC CGA AGG AAC
MAGE-A1	CAG GAG CTG GGC AAT GAA GAC
MAGE-A2	CAT TGA AGG AGA AGA TCT GCC T
MAGE-A2	GAG TAG AAG AGG AAG AAG CGG T
MAGE-A3/6	GAA GCC GGC CCA GGC TCG
MAGE-A3/6	GAT GAC TCT GGT CAG GGC AA
MAGE-A4	CAC CAA GGA GAA GAT CTG CCT
MAGE-A4	TCC TCA GTA GTA GGA GCC TGT
MAGE-A10	CTA CAG ACA CAG TGG GTC GC
MAGE-A10	GCT TGG TAT TAG AGG ATA GCA G
MAGE-A12	TCC GTG AGG AGG CAA GGT TC
MAGE-A12	ATC GGA TTG ACT CCA GAG AGT A

(D)

PCR-primer	sequence (5' - 3')
MAGE-A1	TAG AGT TCG GCC GAA GGA AC
MAGE-A1	CTG GGC AAT GAA GAC CCA CA
MAGE-A2	CAT TGA AGG AGA AGA TCT GCC T
MAGE-A2	CAG GCT TGC AGT GCT GAC TC
MAGE-A3/6	GGC TCG GTG AGG AGG CAA G
MAGE-A3/6	GAT GAC TCT GGT CAG GGC AA
MAGE-A4	CAC CAA GGA GAA GAT CTG CCT
MAGE-A4	CAG GCT TGC AGT GCT GAC TCT
MAGE-A10	ATC TGA CAA GAG TCC AGG TTC
MAGE-A10	CGC TGA CGC TTT GGA GCT C
MAGE-A12	TCC GTG AGG AGG CAA GGT TC
MAGE-A12	GAG CCT GCG CAC CCA CCA A

17. The method of claim 16, wherein primers of group C are used for a first round and/or primers of group D for a second round of PCR-amplification.

18. The method of claim 15 carried out with a single or double pair of PCR-primers amplifying all members of the selected group of MAGE genes, respectively.

19. A diagnostic composition comprising one or more suitable cDNA-primers for simultaneous reverse transcription of more than one different MAGE gene transcripts and optionally an appropriate calibrator mRNA in a single cDNA-synthesis reaction for carrying out the method of any one of the preceding claims.

20. The diagnostic composition of claim 19, wherein at least one cDNA-primer is MgRT3a, Mg1_RT5a or PBGD_RT15b.

21. An oligonucleotide selected from the following group of primers:

MgRT3a

Mg1_RT5a

PBGD_RT15b